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 We use this protocol and it's working

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Isolation of cells from the epithelial layer of frozen human intestinal biopsies

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ABSTRACT

This protocol is for the isolation of the epithelial cells (and associated immune cells within the epithelial layer) from frozen human intestinal pinch biopsies, for use in single-cell sequencing or flow cytometry experiments. This protocol is adapted from Parikh, Antanaviciute, Fawcner-Corbett et al., (2019) Natuer (doi: 10.1038/s41586-019-0992-y). It has been modified specifically with the aim of increasing cell recovery from biopsies of the small intestine.

ATTACHMENTS

[HCA Epithelial cell isolation protocol v1.11.pdf](#)

MATERIALS

- **Transport medium [500ml]** – 490ml DMEM (high glucose), 5ml Pen/Strep, 5ml HEPES (stock = 1M)
- **T5 medium [50ml]** – 47.5ml of Transport Media + 2.5ml FCS
- **HPGA [500ml]** – 500ml HBSS, 5ml Pen/Strep, 5ml HEPES (1M)
- **Wash medium [50ml]** – 50ml HPGA + 1mM EDTA (100ul of 0.5M in 50ml) + 1mM DTT (reconstitute in 100ul of ddH₂O to make 500mM solution, add to 50ml medium to make 1mM)
- **Chelation medium [50ml]** – 50ml HPGA + 1mM EDTA (100ul of 0.5M in 50ml)

Warming media

1 Per sample: pre-warm 10ml of Transport medium in the waterbath.

2 Per sample: pre-warm 20ml of chelation medium in the waterbath.

Collection/Wash

3 Partially thaw frozen biopsies in 37°C waterbath until only a little ice remains.

4 Dropwise add 1ml of pre-warmed transport media to the sample to thaw completely.

5 Use a pastette to transfer the biopsies into the conical of pre-warmed transport media.

6 Place a 70 micron filter onto a 50ml conical.

7 Transfer biopsies onto the filter.

8 Wash with 20ml of cold wash medium.

Crypt isolation

- 9 Transfer biopsies to 5ml warm chelation medium (in 50ml conical).
- 10 Incubate at 37°C for 10 mins (in incubator).
- 11 Vortex 2 x 5-10s.
- 12 Allow biopsies to settle. Remove biopsies from the chelation medium (containing potential isolated crypts) with FCS-coated pastette and transfer to fresh Falcon with 5ml warm chelation medium. Return the biopsies in fresh medium to 37°C incubator and repeat steps 10-12.
- 13 Store chelation medium with the isolated crypts at 4°C while proceeding with subsequent isolation steps.
- 14 Repeat steps 9-13 for a total of 4 times, and pool crypt containing fractions.

Single cell suspension from isolated crypts

- 15 Spin down the pooled crypt suspension (300 g, 5 mins).
- 16 Resuspend in 3 ml warm TrypLE Express with 50 µg/ml DNase at 37°C (75 µl of 2 mg/ml stock).

- 17 Incubate 60 mins at 37°C in incubator shaking incubator (220 RPM).
- 18 Filter with 70 µm cell strainer (prepped with FCS) into 50 ml Falcon, wash through with 5 ml of T5 media.
- 19 Spin filtrate 1500 RPM 5mins.
- 20 Wash pellet with 30ml T5 media.
- 21 Spin 1500 RPM 5mins.
- 22 Very carefully remove supernatant until less than 1ml remains. Resuspend pellet in this.
- 23 Run through 30um cell strainer (prepped with FCS) and wash through with 5ml of PBS.
- 24 Spin 1500 RPM 5 mins, re-suspend in approximately 1ml of remaining PBS, transfer to Eppendorf.

- 25 Make up to 1ml with PBS and take 10 uL for count (1:1 with Trypan blue).
- 26 For 10X single-cell sequencing, count each sample twice using countess to obtain average count and viability:
- 27 Spin down in micro centrifuge (1500 RPM, 5 mins) and re-suspend using live count to concentration of 2000 cells/ μ l (2×10^6 cells/ml).
- 28 re-check count and make volume to 1500 cells/ μ l (1.5×10^6 cells/ml) with PBS.
- 29 Transfer to single cell facility on ice.

Flow cytometry to confirm cell recovery

- 30 Transfer epithelial cells to a round bottom 96 well plate.
- 31 Spin plate at 2200RPM for 2 mins, and dispose of supernatant.
- 32 Add 200 μ l wash buffer, and spin at 2200 RPM for 1 min **x2**.

- 33** Remove supernatant and add 100 µl of FACS buffer.
- 34** Add 1:100 EpCAM-PE (1 µl), 1:50 CD24-PE-CF594 (2 µl) and 1:100 CD45-AF700 (1 µl).
- 35** Place in fridge for 20 mins.
- 36** Spin plate at 2200 RPM for 1 min, and dispose of supernatant.
- 37** Add 200µl wash buffer, and spin at 2200 RPM for 1min **x2**.
- 38** Resuspend in 300µl of FACS buffer and add 1:100 (3 µl) of diluted Sytox Green.
- 39** a.1:60 (5 µl Sytox Green + 295 µl FACS buffer) – final dilution 1:6000.
- 40** 10. Transfer to tubes and run through FACS.